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Biosynthesis of 3-dimethylsulfoniopropionate in marine algae

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13. ABSTRACT *(Maximum 200 words)*

The aim of this research was to investigate the steps in the biosynthesis pathway of 3-dimethylsulfoniopropionate (DMSP) in marine algal groups. DMSP is the biogenic precursor of atmospheric dimethylsulfide (DMS) gas. A combination of in-vivo radioactive and stable isotope labeling and enzyme assay was employed. The pathway was studied in the intertidal green macroalga *Enteromorpha intestinalis* and in three diverse microalgae (*Tetraselmis* sp., *Emiliania huxleyi* and *Melosira nummuloides*. Evidence was obtained for the following pathway in all cases: methionine → 4-methylthio-2-oxobutyrate → 4-methylthio-2-hydroxybutyrate → 4-dimethylsulfonio-2-hydroxybutyrate → DMSP. The enzymes mediating the first three steps, an aminotransferase, a reductase and a methyltransferase, were detected in extracts of *E. intestinalis*. In-vivo 18O-labeling data indicated that the final step is mediated by an oxygenase.

14. SUBJECT TERMS

dimethylsulfoniopropionate, DMSP, biosynthesis, algae

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FINAL REPORT

Grant#: N00014-96-1-0364

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GRANT TITLE: Biosynthesis of 3-Dimethylsulfoniopropionate in Marine Algae

REPORTING PERIOD: 1 Jan 1996 - 1 Jan 1999 (36 months)

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OBJECTIVE: To coordinate a 4-laboratory collaboration (Hanson, Rhodes, Gage, Leustek) to investigate 3-dimethylsulfoniopropionate (DMSP) synthesis in marine algae; to identify the intermediates involved in the macroalga Enteromorpha intestinalis and in phytoplankton species; to begin identifying enzymes of DMSP synthesis; to develop convenient radiochemical syntheses for DMSP and its precursors.

APPROACH: In-vivo radioactive and stable isotope labeling plus mass spectrometry were employed to identify the steps in the pathway. Enzyme assays of algal extracts were used to identify the enzymes mediating these steps. The pathway was studied in the intertidal green alga E. intestinalis and in three diverse microalgae (Tetraselmis sp., Emiliania huxleyi and Melosira nummuloides. [^{35}S]Methionine (Met) was supplied to the algae and labeled metabolites were analyzed by ion exchange chromatography, TLC and electrophoresis. ^{13}C-Labeled Met was supplied with or without $^{18}O_2$, and labeling of metabolites was investigated by mass spectrometric methods. [^{35}S]Met was used to synthesize labeled S-methylmethionine (SMM), 4-methylthio-2-oxo- and 2-hydroxybutyrate (MTOB and MTHB) and 4-dimethylsulfonio-2-hydroxybutyrate (DMSHB). These compounds were used as in-vivo precursors and as enzyme substrates.

ACCOMPLISHMENTS: In collaboration with Rhodes, Gage and Leustek, in-vivo radiotracer and stable isotope labeling experiments were used to define steps in DMSP synthesis, first in E. intestinalis, then in the three phytoplankton species. These steps were shown to be: Met → MTOB → MTHB → DMSHB → DMSP.

We demonstrated that the first three enzymes of DMSP synthesis in E. intestinalis are a 2-oxoglutarate-dependent aminotransferase, an NADPH-dependent reductase, and an S-adenosylmethionine-dependent methyltransferase. We showed that the reductase and methyltransferase are specific for the D-isomer of dimethylsulfoniohydroxybutyrate (DMSHB), and that the aminotransferase has an exceptionally high affinity for methionine (K_m = 30 uM in the presence of 1 mM 2-oxoglutarate). We also showed, in collaboration with Leustek, that DMSHB can act as an osmoprotectant for bacteria.

We developed methods to prepare the following ^{35}S-labeled compounds at

high specific radioactivity, starting from [^{35}S]methionine: DMSP, MTOB, D- and L-MTHB, D- and L-DMSHB, SMM and methylthiopropionate.

CONCLUSIONS: The DMSP synthesis pathway appears to be the same in four diverse algal groups, including important producers of DMS. This pathway is completely different to that in flowering plants, which proceeds via SMM and DMSP-aldehyde. That an aminotransferase with a very high affinity for methionine stands at the head of the algal pathway is consistent with DMSP synthesis competing for methionine with protein synthesis, and may help explain why nitrogen deficiency enhances DMSP production. Depletion of cellular amino acids would favor the transamination reaction, thereby promoting DMSP synthesis when nitrogen is limiting. That DMSHB is an intermediate in DMSP synthesis raises the possibility that catabolism of this compound could contribute to DMS emissions from living algae.

SIGNIFICANCE: This work established for the first time the algal DMSP biosynthesis pathway. Methods for synthesizing isotopically labeled DMSP and intermediates in its synthesis were developed and published.

PUBLICATIONS AND ABSTRACTS (total period of grant):

1. Gage, D.A., Rhodes, D., Nolte, K.D., Hicks, W.A., Leustek, T., Cooper, A.J.L. and Hanson, A.D. (1997) A new route for synthesis of dimethylsulphoniopropionate in marine algae. Nature 387: 891-894

2. Summers, P.S., Nolte, K.D., Cooper, A.J.L., Borgeas, H., Leustek, T., Rhodes, D. and Hanson, A.D. (1998) Identification and stereospecificity of the first three enzymes of 3-dimethylsulfoniopropionate biosynthesis in a chlorophyte alga. Plant Physiology 116: 369-378

3. Cooper, A.J.L. and Hanson, A.D. (1998) Advances in enzymology of the biogeochemical sulfur cycle. Chemtracts - Biochemistry and Molecular Biology 11: 729-747